# nature research

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# Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed | |
|---|---|---|
| ☐ | ☒ | The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ | A description of all covariates tested |
| ☒ | ☐ | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ | A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.* |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Accuri C6 (BD Bioscience) was used to collect flow cytometry data. The LC-MS complete platform consists of an Accela 1250 HPLC system, Accela Open Autosampler, MayLab Mistraswitch column oven and Exactive orbitrap mass spectrometer, controlled by the Xcalibur 3.0.63 software package was used to collect metabolite data. The Thermo Scientific UltiMate 3000 HPLC system using an Acquity UPLC HSS T3 column was used to collect CoQ and CoQH2 data. |
| Data analysis | GraphPad 8 were used for bar graphs output and statistic analysis. FlowJo_V10 was used for flow cytometry data analysis. Raw HPLC-MS data files (Thermo RAW format) were converted to mzXML files for analysis using msconvert, which is part of the ProteoWizard V3 package. The software and algorithms for data analysis used in this study are all well-established from previous work. All software and custom arguments are included in Methods section. There is no unreported algorithm used in this manuscript. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data that support the conclusions in this manuscript are available from the corresponding author upon reasonable request. The source data of immunoblot are provided. The raw data used for generating Figs. 1–4 and Extended Data Figs. 1–9 are included in Source Data files.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences  ☐ Behavioural & social sciences  ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
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| Sample size | All the experiments were performed using sample sizes based on standard protocols in the field. We made an effort to avoid needless use of animals. No statistical tests were used to calculate sample size. In cases where statistics were derived, sample size was n=3 or more independent biological replicates. |
| Data exclusions | For xenograft in vivo experiments, mice were excluded from the analysis if euthanasia had to be applied due to ulcerations tumor growth prior to the end point of the experiment. |
| Replication | Multiple independent repeats were included for related experiments. Each experiment was performed for at least three times to make sure similar results are reproducible. |
| Randomization | For the xenograft studies, 4-6 week female nude mice were chosen as xenograft hosts and randomly allocated into experimental groups. For the PDX models, 4-6 week NSG mice were chosen as xenograft hosts and randomly allocated into experimental groups. |
| Blinding | For cell-based experiments, western blotting and FACS, blinding was not possible because the experiments were performed by a single researcher. Mass spectrometry analysis were blinded to group allocation during data collection. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology |
| ☐ | ☒ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

| | |
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| Antibodies used | DHODH (1:1,000, 14877-1-AP, Proteintech), GPX4 (1:1,000, MAB5457, R&D systems), Vinculin (1:3,000, V4505, Sigma-Aldrich), SLC7A11 (1:1,000, 12691, Cell Signaling Technology), ACSL4 (1:1,000, sc-271800, Santa Cruz), COQ2 (1:1,000, sc-517107, Santa Cruz Biotechnology), FSP1 (1:1,000, sc-377120, Santa Cruz Biotechnology), Flag (1:10,000, 66008-3-Ig, Proteintech), 4-HNE (1:400, ab46545, Abcam), Ki67 (1:500, 9027S, Cell Signaling Technology) and Cleaved Caspase-3 (1:500, 9661S, Cell Signaling Technology). All antibodies were diluted n TBST buffer containing 3% BSA. |
| Validation | All antibodies used in our study have been validated and detailed information could be found on the website from manufactures as listed below. Some of them have also been validated by our experiments as shown in this manuscript using either over-express, knockout or knockdown strategies.<br>DHODH: https://www.ptglab.com/products/DHODH-Antibody-14877-1-AP.<br>GPX4: https://www.rndsystems.com/products/human-mouse-rat-glutathione-peroxidase-4-gpx4-antibody-565320_mab5457.<br>Vinculin: https://www.sigmaaldrich.com/catalog/product/sigma/v4505?lang=en&region=US.<br>SLC7A11: https://www.cellsignal.com/products/primary-antibodies/xct-slc7a11-d2m7a-rabbit-mab/12691.<br>ACSL4: https://www.scbt.com/p/acsl4-antibody-a-5.<br>COQ2: https://www.scbt.com/p/coq2-antibody-2b4.<br>FSP1: https://www.scbt.com/p/amid-antibody-b-6.<br>Flag: https://www.ptglab.com/products/Flag-tag-Antibody-66008-3-Ig.<br>4-HNE: https://www.abcam.com/4-hydroxynonenal-antibody-ab46545.<br>Ki67: https://www.cellsignal.com/products/primary-antibodies/ki-67-d2h10-rabbit-mab-ihc-specific/9027. |

Cleaved Caspase-3: https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-antibody/9661.

# Eukaryotic cell lines

Policy information about cell lines

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| Cell line source(s) | UMRC2, UMRC6, and RCC4 cell lines were provided by W. G. Kaelin at Dana-Farber Cancer Institute. TK-10 cell line was obtained from Dr. Gordon Mills at MD Anderson Cancer Center. HT-1080, NCI-H226, A498, SW620, U-87 MG, A549, NCI-H1437, MDA-MB-436, MDA-MB-231, 786-O, 769-P, HCT-8, 294T, Hela, Jurcat were obtained from the American Type Culture Collection. |
| Authentication | Cell line were not authenticated. |
| Mycoplasma contamination | All cell lines tested negative for mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | No ICLAC cell line was used in this study. |

# Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| | |
|---|---|
| Laboratory animals | Athymic nude and NOD scid gamma (NSG) female mice at 4-6 week old were purchased from ERO mouse facility in MD Anderson Cancer Center. Rodent housing conditions used in this study are: temperature set point: 72F; high limit: 74F; low limit: 70F. Humidity set point: 45%; high limit: 55%; low limit: 40%. Light cycle: 12 hour light/dark. |
| Wild animals | No wild animals involved in this study. |
| Field-collected samples | This study didn't involve samples collected from field. |
| Ethics oversight | All the xenograft model experiments were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee and Institutional Review Board at The University of Texas MD Anderson Cancer Center. The study is compliant with all relevant ethical regulations regarding animal research. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Flow Cytometry

## Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

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| Sample preparation | Cells cultured in 6-well plates were incubated with media containing 4 µM lipid peroxidation dye CM-H2DCFDA (Life Technologies, #C6827) for 30 minutes at 37℃. Then cells were trypsinized and collected into 1.7 ml tubes. After washed once with PBS, cells were resuspended in cold PBS and subjected to flow cytometry analysis. For cell death measurement, cells were stained in PBS with PI for 5 minutes before flow cytometry analysis. |
| Instrument | Accuri C6 (BD Bioscience) |
| Software | Using Accuri C6 software to collect data and FlowJo_V10 software to analyze data. |
| Cell population abundance | At least 5000 cells were analyzed for each sample. |
| Gating strategy | Initial cell population gating (FSC-Area Vs FSC-Height) was adopted to make sure doublet exclusion and only single cell was used for analysis. A figure exemplifying the gating strategy would be provided upon request. |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.